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Effects of Phosgene Exposure on Bacterial, Viral, and Neoplastic Lung Disease Susceptibility in Mice

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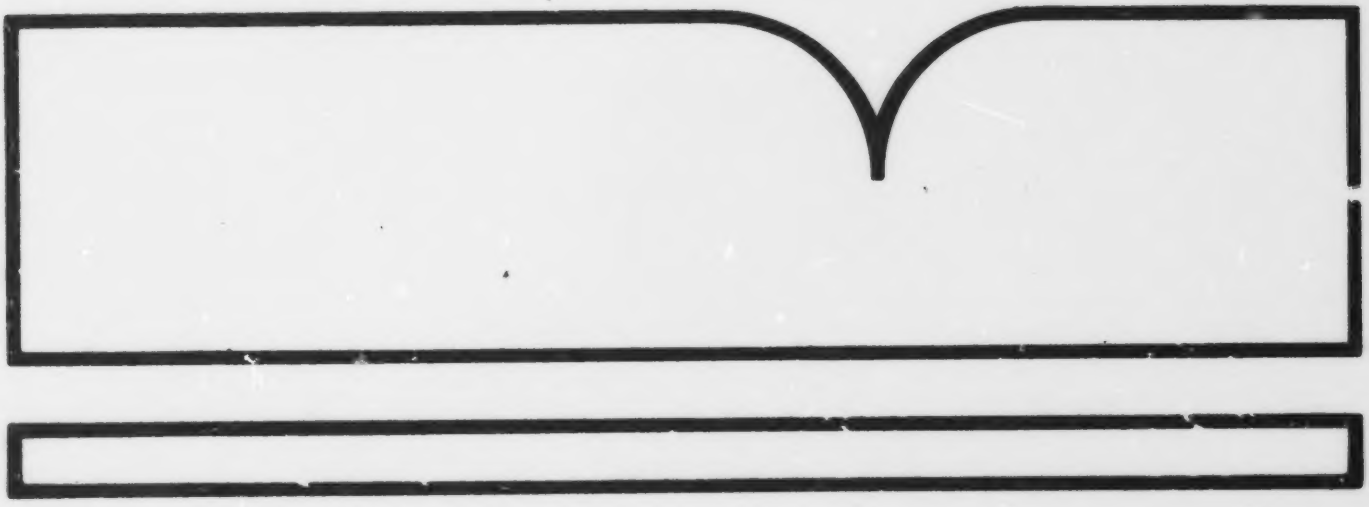

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16. ABSTRACT

The effects of phosgene inhalation exposure on host resistance models representative of bacterial, viral, and neoplastic lung diseases were assessed. A single 4 h exposure to concentrations of phosgene of 0.025 ppm and above significantly enhanced mortality due to aerosol infection with Streptococcus zooepidimicus and significantly increased the number of B16 melanoma tumors which developed in the lungs of mice following intravenous inoculation of syngeneic tumor cells. In contrast, 5 daily 4 h exposures to 0.5 ppm phosgene did not affect mortality following infection with influenza virus. Bacteria recovered from lavage fluid of mice exposed to 0.05 ppm for 4 h increased between 3 and 48 h post infection while bacteria recovered from lavage fluid of air-exposed mice declined to nearly undetectable levels. Concentrations of phosgene 10-fold higher than the lowest observable effect concentration for streptococcus susceptibility had little or no effect on alveolar macrophage phagocytic activity or on total cells recovered, viability, or differential cell counts in lavage fluid obtained shortly after exposure. When exposure time was increased from 4 to 8 h, significant effects on mortality due to streptococcus infection were observed at 0.01 ppm but effect on the B16 tumor model were not observed below 0.025 ppm in phosgene. The data indicate that exposure to phosgene concentrations equal to 1-25% of the current TLV (0.1 ppm) significantly enhanced susceptibility to bacterial and tumor disease in mice.

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EFFECTS OF PHOSGENE EXPOSURE ON BACTERIAL, VIRAL, AND NEOPLASTIC LUNG DISEASE SUSCEPTIBILITY IN MICE

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In this study the effects of phosgene inhalation on host resistance models representative of bacterial, viral, and neoplastic lung diseases were assessed. A single 4-h exposure to concentrations of phosgene of 0.025 ppm and above significantly enhanced mortality due to laboratory-induced aerosol infection with Streptococcus zooepidimicus *(group C). Bacteria recovered from lavage fluid of mice exposed to 0.05 ppm for 4 h increased dramatically between 3 and 48 h post infection, while bacteria recovered from lavage fluid of air exposed mice declined to nearly undetectable levels over the same time period. Concentrations of phosgene 10-fold higher than the lowest observable effect concentration for streptococcus susceptibility had little or no effect on alveolar macrophage phagocytic activity and little or no effect on total cells recovered, viability, or differential cell counts in lavage fluid obtained shortly after exposure. A single 4-h exposure to as little as 0.025 ppm phosgene also caused a significant increase in the number of B16 melanoma tumors that developed in the lungs of mice following intravenous inoculation of syngeneic tumor cells. In contrast, 5 daily 4-h exposures to 0.5 ppm phosgene had no effect on mortality following infection with influenza virus and only minimal effects on lung virus titers. When exposure time was increased from 4 to 8 h, significant effects on mortality due to streptococcus infection were observed at an exposure concentration of 0.01 ppm. However, increasing the exposure time from 4 to 8 h did not alter the lowest observable effect concentration (0.025 ppm) for the B16 tumor model. The data indicate that exposure to phosgene concentrations equal to 10–25% of the current threshold limit value (0.1 ppm) significantly enhanced susceptibility to bacterial and tumor disease in mice. In contrast, the concentrations of phosgene used in this study had little effect on the influenza virus infection.*

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

The authors gratefully acknowledge Margaret Ménache and Donald Doerfler for statistical analyses, Thomas Williams, Margaret Beaman, and Robert Gluck for excellent technical support, and Drs. Gary Hatch and Hillel Koren for providing useful insights during the course of the study.

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INTRODUCTION

Phosgene ($COCl_2$), a volatile acid chloride used as a chemical warfare agent during World War I, is now widely used by industry in the synthesis of isocyanates (which are the starting materials for polyurethane resins) and to a lesser degree in the production of polycarbonates, pesticides, and dye intermediate products. NIOSH (1976) estimated that approximately 10,000 workers were potentially exposed to phosgene during its manufacture and use. The threshold limit value (TLV) is currently 0.1 ppm for an 8-h exposure. Phosgene is also present in ambient air (at concentrations as high as 61 parts per trillion), where it is probably generated during oxidation of halogenated compounds or during photodecomposition of chloroethylenes (Singh, 1976).

Some of the effects produced by phosgene inhalation are similar to effects produced by inhalation of ozone (O_3) and nitrogen dioxide (NO_2). All three are classified as lower-respiratory-tract irritants because they cause characteristic alterations in the breathing patterns of mice (Alarie, 1973). The effects of phosgene on induction of pulmonary edema, lung sulfhydryl and glucose-6-phosphate dehydrogenase content, and lung histopathology are all similar to effects produced by O_3 and NO_2 (Franch and Hatch, 1986), and like O_3 and NO_2, phosgene increases sodium pentobarbital-induced sleeping time (Illing et al., 1988). O_3 and NO_2 increase susceptibility to bacterial infection of the lung at very low exposure concentrations relative to other indicators of toxicity (Gardner, 1982), and certain O_3 exposure regimens enhance susceptibility to influenza in mice (Selgrade et al., 1988). Thus, it was hypothesized that phosgene might also alter susceptibility to these infections as well as susceptibility to B16 melanoma tumors in the lung following intravenous (IV) injection of mice with syngeneic tumor cells, a tumor model first described by Fidler and Nicolson (1976). An increase in B16 melanoma tumors in the lung has been reported following exposure to NO_2 but could not be demonstrated following O_3 exposure (Richters, 1988).

Highly reactive inhaled compounds such as phosgene, which does not appear to have significant extrapulmonary circulation (Slade et al., 1983), present special problems in terms of assessing effects on susceptibility to infectious and neoplastic disease. Most immunotoxicity testing is done by assessing the function of spleen cells in animals (Luster et al., 1988). However, immune cells in the respiratory tract (which with the exception of macrophages are more difficult to obtain than those in the spleen) represent the first line of defense against infectious agents that enter the host via inhalation and against tumors initiated in or metastasized to the lung. Inhaled compounds may affect the function of these cells and hence susceptibility to respiratory disease without altering spleen cell function. In addition, a number of innate host resis-

tance factors (e.g., the struction of the lung and products secreted by the mucous membrane) exist that may be altered by inhaled compounds and affect susceptibility to infectious or neoplastic disease.

One solution to these problems is to assess the effects of such inhaled compounds on several host resistance models in mice that are representative of major types of human respiratory disease. We have taken this approach by determining the effects of phosgene exposure on susceptibility of mice to bacterial, viral, and neoplastic disease models in the lung. Exposure regimens tested were ones that in our previous experience with O_3 had enhanced susceptibility to a particular infection. Hence, a single exposure followed by infection was used to determine effects on the streptococcus infectivity model, and five repeated daily exposures with infection after the second exposure were used to determine effects on the influenza infectivity model. Because we had little previous experience with the B16 melanoma tumor model, both types of exposure regimens were tested.

MATERIALS AND METHODS

Mice

CD-1 female mice were obtained from Charles River (Kingston, NY, or Raleigh, NC). C57BL/6 female mice were obtained from Jackson Laboratories, Bar Harbor, ME, and were 8–12 w old at the time of use. Randomly selected mice tested serologically on arrival and sentinel mice monitored serologically throughout the study were free of Sendai virus, pneumonia virus of mice, mouse hepatitis virus, and a number of other murine viruses, as well as mycoplasma. Mice were also monitored for and found to be free of ectoparasites and endoparasites. The numbers of mice included in each experiment are indicated on the individual tables and figures.

Exposure

Mice in individual cages were exposed to either phosgene or filtered air within an 11.4-ft^3 Rochester-type chamber. Phosgene, obtained as a mixture in nitrogen (Matheson Gas Products, Morrow, GA), was added to filtered room air before the metering orifice of the chamber at a flow rate of 1 chamber volume/min. A Tylan mass flow controller was used to meter the phosgene into the airstream, and the chamber air was sampled at least every 10 min. Phosgene concentrations in the chamber were determined with a Hewlett Packard 11831 gas chromatograph containing a 6 ft × 1/8 in Teflon column filled with Chromosil 310 packing (Supelco Incl., Bellefonte, PA) operating at 70°C (Esposito et al., 1977). Methane (5%) in argon was used as the mobile phase at a flow rate of 43 cm^3/min. Phosgene was detected by electron capture. The gas

chromatograph was calibrated at least bimonthly with a permeation tube containing liquid phosgene (Analytical Instrument Development Inc., Avondale, PA). A Wilks Miran 80 infrared analyzer was used as a second check of the phosgene concentration. Standard deviations of the mean for actual chamber concentrations were never more than 10% of the stated concentrations. Temperature within the chambers was maintained between 71 and 77 °F, and relative humidity was maintained between 50 and 70%. Except where otherwise indicated, exposures were for 4 h. Mice were not exposed to levels higher than 0.5 ppm because higher exposure levels caused significant mortality in the absence of any other treatment.

Streptococcal Infectivity Model

Immediately after a single 4-h air or phosgene exposure (except where otherwise indicated) 5-wk-old mice from both exposure groups were placed in a 11.4-ft ^{3} stainless steel Rochester-type chamber under negative pressure and exposed to an aerosol of viable *Streptococcus zooepidemicus* (group C). Approximately 2×10^9 organisms in 4 ml from a 24-h culture grown in Todd-Hewitt Broth were placed in a DeVilBiss no. 40 nebulizer and aerosolized over a period of 20 min. Immediately following all bacterial exposures, one mouse from each exposure group was sacrificed in order to determine the total number of bacteria deposited into the lungs at time zero. Lungs were aseptically excised and homogenized in sterile Todd-Hewitt broth. Aliquots were appropriately diluted (so as to obtain 10–100 cfu/plate) and inoculated on blood agar plates in duplicate. The number of colony-forming units (cfu) on the plates was determined following a 24-h incubation. For the experiments reported here the number of bacteria deposited ranged from 300 to 6800 cfu/lung. Previous studies indicated that within the range of 200–4000 cfu, the ensuing mortality in controls did not vary as a function of cfu deposited (Miller et al., 1978). Where indicated, similar methods were used to determine the number of bacteria in lung homogenates or lung lavage fluid at various times post infection. Animals to be observed for percent mortality and mean survival time (MST) were held for 15 days. MST was calculated using the following formula:

$$\text{MST} = \frac{\left[\sum_{j=1}^{15} j\beta j\right]}{N}$$

where β_j is the number of mice surviving j days, N is the total number of mice in the group, and 15 represents the duration of the experiment.

In order to determine whether phosgene altered the ability of alveolar macrophages to phagocytize bacteria, mice were anesthetized with sodium pentobarbital following a single 4-h air or phosgene exposure and injected intratracheally (IT) with approximately 5×10^7 streptococci suspended in 0.05 ml saline using methods previously described by Hatch et al. (1981). After 30 min, during which mice slept on their backs, mice were killed with an overdose of sodium pentobarbital, the trachea was exposed, a catheter was inserted, and lungs were flushed 6 times with 35 ml/kg body weight saline warmed to 37°C. The first lavage fluid remained in the lung for 3–5 min; subsequent lavages were removed immediately. Cell pellets were obtained by centrifugation, resuspended in saline, and divided into aliquots. Trypan blue to a final concentration of 0.1% was added to one aliquot, and total cell counts and viability were determined using a hemacytometer. Differential cell counts and percent phagocytosis were done on two 0.50-ml aliquots on duplicate microscope slides using a cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, PA) and Diff-Quik stain kit (American Scientific Products, McGraw Park, IL). A total of 100 intact macrophages per duplicate slide was counted.

Influenza Infectivity Model

Influenza A/HongKong/68 (H3N2) was prepared from mouse lung extracts and assessed for plaque-forming units (pfu) on Madin Darby canine kidney (MDCK) cell monolayers as previously described (Selgrade et al., 1988). CD-1 mice 3–4 wk old were exposed to phosgene or air 4 h/day for 5 consecutive days and infected with the virus after the second of these exposures. Infection was carried out by anesthetizing mice with ether and inoculating intranasally 75 pfu of virus in 0.05 ml Hanks balanced salt solution (HBSS). Either mice were held and observed for mortality, or lungs were removed on various days post infection (PI) and wet weight and virus titers in 10% lung extracts were determined as previously described (Selgrade et al., 1988).

B16 Tumor Model

B16/BL6 melanoma tumor cells, obtained from Dr. Robert Wiltrout (National Cancer Institute, Frederick, MD), were passed several times in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% nonessential amino acids, 1% L-glutamine, and 1% 100 × MEM vitamins (GIBCO Laboratories, Grand Island, NY). A stock pool of cells was created after approximately 8 passages in our laboratory and stored in 10% DMSO at −70°C. For each experiment cells from this stock pool were thawed and grown to confluency in 150-cm^2 flasks. On the day of injection cells were removed from the flask with 0.25% trypsin/1 m*M* EDTA (GIBCO Laboratories, Grand Island, NY), held in growth medium for 45 min, and resuspended in phosphate-buffered

saline at a concentration of 1.25×10^5 cells/ml. C57BL/6 mice were injected IV with 0.2 ml of the cell suspension in the lateral tail vein either 1 day after a single phosgene or air exposure or after the second of five 4-h daily phosgene or air exposures. Lungs were removed 2-3 wk post injection and teased apart, and tumors, which appeared as black spots, were counted. Lungs that did not have any tumors were considered to be the result of failure of the IV injection and were eliminated from both the phosgene and air groups. The number of lungs eliminated for this reason usually represented a small percentage of lungs removed and did not vary as a function of treatment.

Statistics

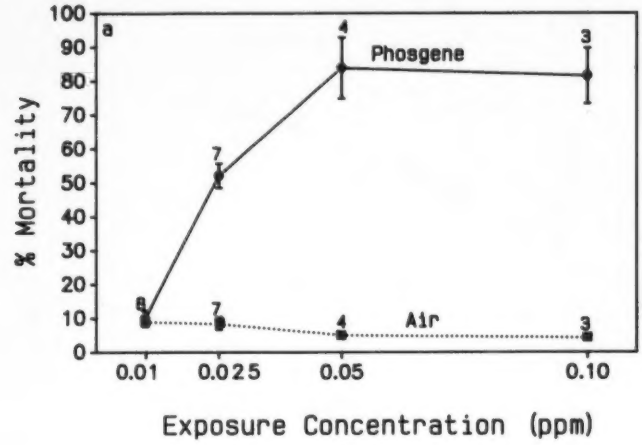
Data were analyzed using analysis of variance models appropriate for the various types of experimental studies that were performed as indicated in figure and table legends. Where assumptions required for ANOVA were not met, nonparametric procedures were used. A probability level of .05 or less was considered to be indicative of significant difference from control.

RESULTS

Effects on Susceptibility to Streptococcal Infection

Mortality due to streptococcal infection was significantly enhanced following exposure to 0.025 ppm phosgene and was maximally enhanced following exposure to 0.05 ppm phosgene (Fig. 1*a*). Mean survival time was maximally decreased following exposure to 0.05 ppm phosgene, and was inversely related to exposure concentration at levels below 0.05 ppm. (Fib. 1*b*). When exposure to the bacterial aerosol was delayed until 3 h after exposure to 0.05 ppm phosgene, there was still significant enhancement of mortality (30 dead out of 40 phosgene-exposed vs. 1 dead out of 40 air-exposed). However, when exposure to the bacterial aerosol was delayed for 24 h there was no difference between phosgene and air exposed mice (0 dead out of 40 in both exposure groups).

Figure 2 shows recovery of bacteria from lavage fluid at various times PI in mice exposed to 0.05 or 0.025 ppm phosgene compared to air-exposed mice. For both exposure concentrations there was a significant time by exposure treatment interaction. Similar results were obtained for bacteria recovered from whole lung homogenates. In mice exposed to air most bacteria were cleared from the lung within 3–24 h PI. Significantly more bacteria (300–400 cfu) were recovered immediately after infection of mice exposed to 0.05 ppm phosgene compared to air controls (Fig. 2*a*). Following 0.05 ppm phosgene exposure there was a significant decrease in bacteria recovered from lavage fluid be-



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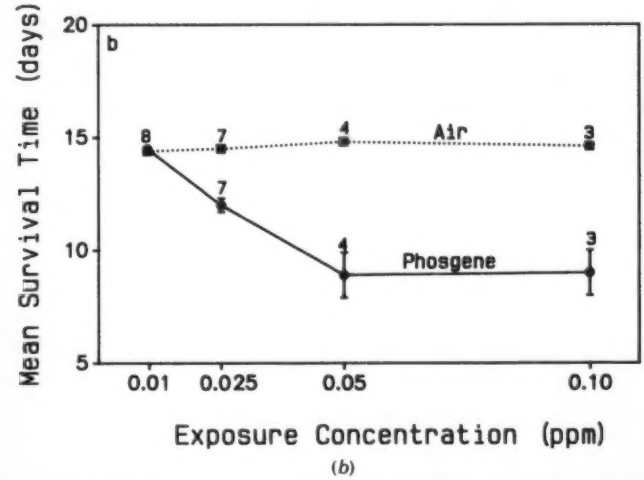

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FIGURE 1. Effect of 4-h phosgene exposure on susceptibility to streptococcus infection. Data points represent the (a) mean percent mortality or (*b*) mean survival time and standard error for the number of experimental replicates indicated above the error bar. (Standard error for air-exposed mice is too small to be visible on the graph in some cases.) Each experimental replicate included 40 mice per exposure group. Data were analyzed using one-way analysis of variance (ANOVA). Significant ANOVA effects were subtested using Duncan's multiple range procedure.

tween 0 and 3 h PI although the amount of bacteria recovered at this time was significantly higher than that recovered from air controls. From 3 to 48 h PI there was a steady increase in the amount of bacteria recovered from phosgene-exposed mice in contrast to air controls, where little or no bacteria was detectable at 48 h PI. Following exposure

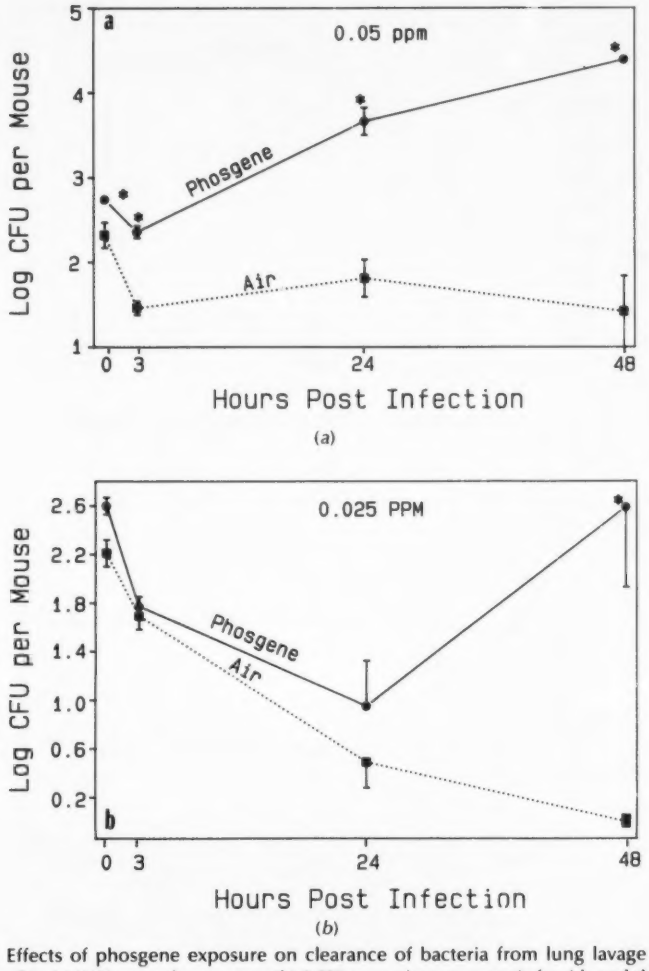


FIGURE 2. Effects of phosgene exposure on clearance of bacteria from lung lavage fluid. Mice were exposed to (*a*) 0.05 ppm phosgene or (*b*) 0.025 ppm phosgene or air for 4 h and then immediately infected by aerosol with streptococci. Lavage was collected at various times post infection and the number of colony-forming units (cfu) of bacteria per lavage was assessed on blood agar plates. Data points represent the mean and standard error for 5–10 mice. Asterisk indicates significantly different from air controls ($p < .05$) using two-way ANOVA and nonparametric procedures.

to 0.025 ppm phosgene there was no difference in the number of bacteria recovered at time zero from phosgene or air-exposed mice (Fig. 2*b*). There was a steady decline in the number of bacteria recovered from phosgene-exposed mice from 0 to 24 h PI. However, at 48 h PI there was a significant increase in bacteria recovered. Significant differences be-

tween air and 0.025 ppm phosgene-exposed mice were not observed until 48 h PI.

In order to assess the effects of phosgene exposure on macrophage phagocytic activity, mice were infected intratracheally immediately after air or phosgene exposure with a much larger number of bacteria than could be introduced by inhalation. This was done to enhance the likelihood that all macrophages would have the opportunity to phagocytize bacteria. Table 1 shows that phosgene exposure concentrations 10 times those that caused significant increases in susceptibility to streptococci had no significant effect on the percent of macrophages that ingested streptococci. The only exception was the 0.4 ppm exposure group, for which at least 95% of macrophages from phosgene-exposed mice were phagocytic. There were also no significant differences in the number of bacteria per macrophage in the air- and phosgene-exposed groups. The vast majority of macrophages in each group contained more than three bacteria (data on number of bacteria per cell not shown). There was also very little difference in total and differential cell counts and in cell viability between air- and phosgene-treated mice immediately after exposure.

Effects on Susceptibility to Influenza Infection

Exposure to 0.5 ppm phosgene for 5 consecutive days had no effect on influenza-induced mortalities in mice infected after the second of the 5 exposures (4 dead as a result of influenza infection out of a total of 15 exposed to either air or phosgene). Figure 3*a* shows the effect of 0.5 ppm phosgene exposure on lung wet weight following influenza infection. There was no statistically significant effect on the wet weight

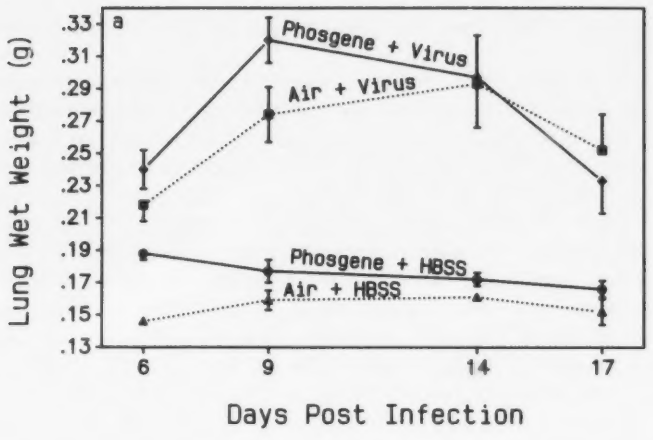
TABLE 1. Effects of Phosgene on Lavage Fluid Cells[a]

Phosgene concentration (ppm)	Total cells ($\times 10^5$)	Percent viable	Percent macrophages	Percent PMN	Percent lymphocytes	Percent phagocytic macrophages
0.0	4.7 ± 0.9	90.5 ± 1.6[b]	95.8 ± 0.8	1.1 ± 0.4	2.9 ± 0.4	97.8 ± 1.1
0.25	4.3 ± 0.6	90.8 ± 1.0	94.6 ± 1.3	1.7 ± 0.6	3.6 ± 0.8	97.7 ± 1.4
0.0	6.4 ± 0.7	95.6 ± 0.6	96.5 ± 0.6	1.3 ± 0.3	2.2 ± 0.4	100.0 ± 0.0
0.4	6.2 ± 0.6	92.1 ± 0.8[c]	93.8 ± 1.3	3.3 ± 0.7[c]	2.9 ± 0.6	95.1 ± 0.8[c]
0.0	7.5 ± 0.7	85.2 ± 1.3	96.6 ± 0.7	1.4 ± 0.3	2.0 ± 0.4	99.9 ± 0.1
0.5	5.6 ± 0.4[c]	74.3 ± 2.4[c]	92.7 ± 1.6[c]	2.9 ± 0.5[c]	3.5 ± 0.6	97.7 ± 0.8

[a]There were fewer than 1% eosinophils in all of the samples examined.

[b]Mean ± SE for 9–15 mice; mice were exposed to air or phosgene for 4 h, anesthetized, and injected IT with 5×10^7 bacteria. After 30 min lavage fluid was collected.

[c]Significantly different from air control ($p < .05$) using one-way analysis of variance for individual experiments.



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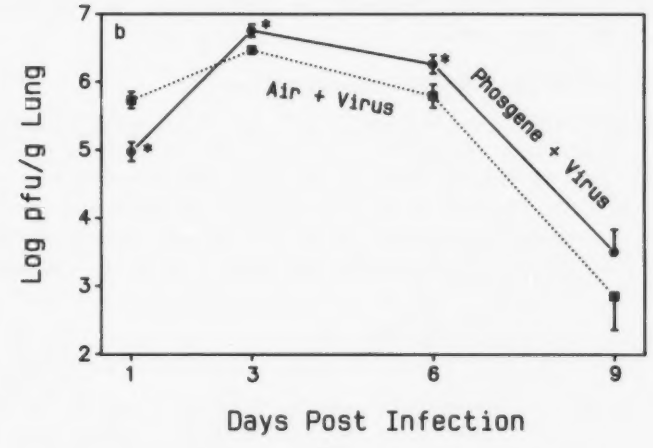


(*b*)

FIGURE 3. Effects of phosgene exposure of lung wet weight and lung virus titers in mice infected with influenza virus. Mice were exposed daily to air or 0.5 ppm phosgene for 5 days, 4 h/day, and influenza virus or Hanks balanced salt solution (HBSS) was instilled intranasally after the second exposure. Lungs were removed on various days post infection, (*a*) wet weight was assessed, and (*b*) virus titers in 10% lung homogenates were determined for virus-infected mice. Data points represent the means and standard errors for four to five mice. Asterisk indicates significantly different from air control ($p < .05$) using three-way ANOVA and $\log_{10}$-transformed data for wet weight data and two-way ANOVA and nonparametric procedures for virus titers.

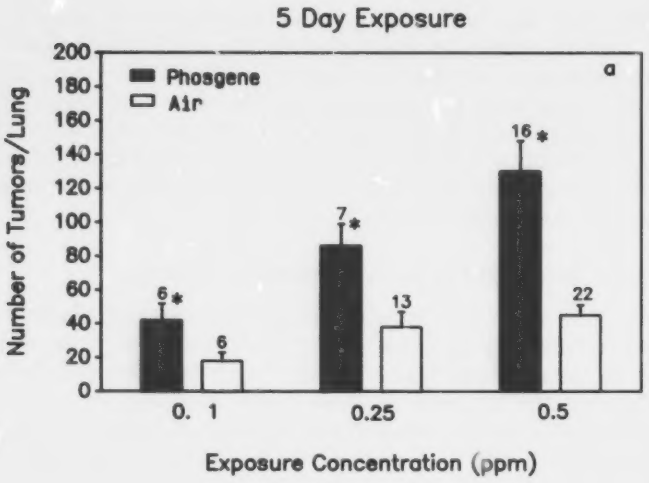
of infected mice exposed to phosgene, although wet weights 6 and 9 days PI were qualitatively higher in phosgene-exposed mice. When lung virus titers were assessed in similarly treated mice on various days PI (Fig 3*b*), there was a significant gas by day interaction. Titers in phosgene-treated mice were significantly lower 1 day PI and significantly higher 3 and 6 days PI compared to air controls; however, all of these differences were less than 1 log. There was no difference between the two treatment groups 9 days PI.

Effects on Susceptibility to B16 Melanoma Tumors

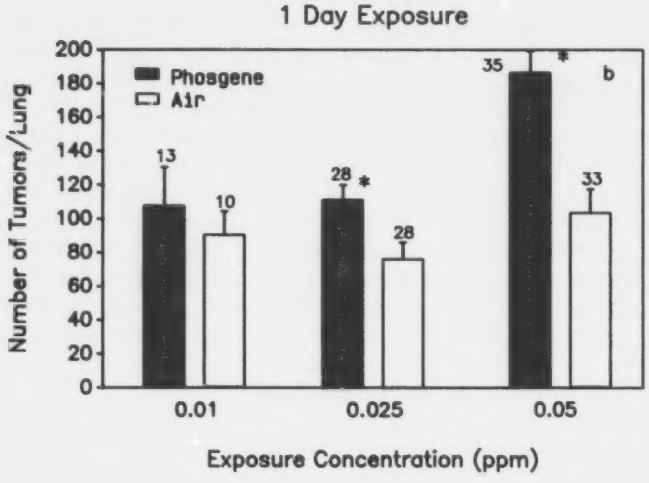
Initially, the effect of phosgene exposure on susceptibility to B16 melanoma tumors was assessed by exposing mice to air or phosgene, 4 h/day, for 5 consecutive days and injecting the tumor cells intravenously following the second of those exposures. Figure 4*a* shows that there was a significant increase in the number of lung tumors observed in mice exposed to 0.1, 0.25, and 0.5 ppm phosgene. In order to determine the relative importance of the initial exposure to phosgene that preceded tumor inoculation relative to subsequent exposures, mice were divided into four groups. The groups received a single air or 0.5 ppm phosgene exposure and were inoculated with tumors the following day. The remaining 2 groups were inoculated with tumors and exposed to phosgene or air on the same day and on 3 subsequent days. Table 2 shows that there was a significant increase in the number of tumors observed in mice exposed to phosgene 1 day prior to tumor inoculation. However, although the number of tumors in mice exposed to phosgene on 4 consecutive days beginning immediately after tumor inoculation was qualitatively higher than the number of tumors in air controls, the difference was not statistically significant. Hence, remaining experiments to test lower exposure concentrations employed a single exposure with tumor inoculation the following day. Figure 4*b* shows that a single exposure to 0.05 ppm and 0.025 ppm caused significant increases in the number of lung tumors observed; however, no increase was observed in mice exposed to 0.01 ppm.

Effects of 8-h Exposure to Phosgene

Effects of an 8-h exposure to 0-.01 ppm phosgene were assessed in order to determine whether extending the exposure from 4 to 8 h would result in significantly enhanced susceptibility to streptococci and B16 tumors at a concentration for which significant effects had not been previously observed. Table 3 shows that susceptibility to streptococci was significantly enhanced by exposure to 0.01 ppm phosgene; however, susceptibility to B16 tumors was not enhanced by this exposure. It should be noted that an 8-h exposure to 0.025 ppm phosgene significantly enhanced susceptibility to B16 tumors. Exposure concen-



(a)



(b)

FIGURE 4. Effects of phosgene on susceptibility to B16 melanoma tumors. Mice were (a) exposed to air or phosgene daily for 5 days, 4 h/day, and injected intravenously with tumors after the second exposure, or (*b*) given a single exposure to air or phosgene and injected with tumors on the following day. Lungs were removed 2–3 wk after tumor cells were injected, and tumors were counted. Data points represent the means and standard error for the number of mice indicated over the error bar. Asterisk indicates significantly different from air control ($p < .05$). Each concentration level was analyzed independently using one-way ANOVA and nonparametric procedures.

TABLE 2. Effects of Phosgene Exposure Regimen on B16 Tumors

	Number of tumors/lung[b]	
Exposure regimen[a]	0.5 ppm Phosgene	Air
1 Day prior to tumors	96 ± 11 (n = 16)[c]	38 ± 2 (n = 18)
4 Days after tumors	65 ± 8 (n = 11)	48 ± 6 (n = 15)

[a]Mice were exposed to phosgene for 4 h and injected with the tumors on the following day or injected with tumors and then exposed to phosgene for 4 h/day for 4 days.
[b]Means ± SE for number of mice indicated in parentheses.
[c]Significantly different from air control ($p < .05$) using two-way ANOVA.

trations of phosgene below 0.01 ppm could not be tested because of limitations in our generating and monitoring capabilities.

DISCUSSION

A single 4-h exposure to phosgene concentrations as low as 0.025 ppm significantly enhanced mortality due to streptococcal infection in mice and significantly enhanced the number of tumors that developed in the lungs of mice following IV injection of B16/BL6 melanoma cells. In contrast, the highest tolerable concentration of phosgene (0.5 ppm) had no effect on influenza-induced mortalities or lung wet weight in influenza-infected mice and only minimal effects on the amount of influenza virus recovered from the lungs. When the exposure time was increased from 4 to 8 h, a significant increase in susceptibility to streptococcus was also seen at an exposure concentration of 0.01 ppm; however, 0.01 ppm exposure for 8 h did not result in increased susceptibility

TABLE 3. Effects of 8-Hour Exposure

	Disease susceptibility	
Exposure[a]	Streptococci, number dead/total (% mortality)	B16 lung tumors, number tumors/ lung ± SE
0.01 ppm Phosgene	24/80 (30%)[b]	9 ± 3 (n = 7)
Air	3/80 (4%)	9 ± 3 (n = 10)
0.025 Phosgene	ND[c]	25 ± 4 (n = 12)[b]
Air	ND	6 ± 2 (n = 12)

[a]Mice were exposed to phosgene or air for 8 h and either infected immediately with streptococci or injected on the following day with B16 tumor cells.
[b]Significantly different from air control ($p < .05$), using one-tailed Fisher's exact test for mortality and one-way ANOVA for tumors.
[c]ND = not done.

to B16 tumors. The effects of phosgene on the streptococcus and melanoma tumor models are particularly important because; they occurred at levels 4- to 10-fold below the current TLV. Following a single 4-h exposure, Currie et al. (1978) reported that an increase in PMNs represented the most sensitive cellular indicator of phosgene toxicity with significant effects occurring after exposure to 0.25 ppm. Significant increases in lavage fluid protein content in mice, hamsters, and rats have been reported following a single 4-h exposure to 0.2 but not 0.1 ppm phosgene (Hatch et al., 1986). To our knowledge, significant effects following exposure to concentrations lower than 0.2 ppm have not been previously reported. Hence the streptococcus and B16 melanoma models are 10-fold more sensitive to the effects of phosgene than any of the other end points that have been monitored.

The mechanisms responsible for increased sensitivity to both bacterial infection and tumor development are unclear. Because similar levels of sensitivity were observed with both models, some defense mechanism(s) common to both diseases may have been affected. Alternately, different but equally sensitive defenses may have been affected, possibly by some common mecahnism(s). The alveolar macrophage plays an important role in clearing bacteria from the lung (Green and Kass, 1964). However, phagocytic capabilities of these cells were largely unaffected by phosgene exposure concentrations 10-fold higher than those that produced effects on the two disease models. Given that the maximum number of bacteria deposited in the lung following inhalation of streptococci was never more than 7000 and that following phosgene exposure at least 95% of the more than 100,000 macrophages removed from the lung were phagocytic, there would appear to be ample phagocytic capacity. Because we did not assess macrophage bactericidal activity, it is possible that engulfed bacteria were not inactivated. It is also possible that phosgene somehow inhibited macrophage chemotaxis and hence prevented macrophages from mobilizing to the sites of infection in the lung. Although the alveolar macrophage has been shown to have tumoricidal activity in some situations (Sone and Fidler, 1980), in the case of the B16 model, the macrophage has been shown to suppress tumoricidal activity of natural killer cells thought to be important in preventing tumor metastases (Parhar and Lala, 1985). Elimination of this suppressor activity, which is mediated by prostaglandin E_2 (PGE_2), with indomethacin is beneficial in terms of containing the tumors. Hence, the alveolar macrophage does not appear to provide some common thread between the streptococcus and B16 disease models (as a result of killing both bacteria and tumor cells) that might explain their sensitivity to phosgene. However, O_3 increases PGE_2 levels in both human and rat lavage fluid (Seltzer et al., 1986; Giri et al., 1981). If phosgene were to have similar effects on PGE_2 levels or on some other immune modula-

tor, such effects could account for the increased susceptibility in both disease models by altering natural killer cell activity against B16 tumors and alveolar macrophage responses to bacteria.

Several other factors might account for increased sensitivity to streptococci. Following exposure to 0.05 ppm phosgene, significantly more bacteria were deposited in the lung compared to the air group (Fig. 2). Similar differences have been observed following ozone exposure (Miller et al., 1978). It is unlikely that this increase in the initial number of bacteria deposited could account for the increased susceptibility observed. Miller et al. (1978) demonstrated that there was no relationship between the number of bacteria deposited (within the range from 200 to 4000 cfu) and percent mortality. Host defenses in phosgene-exposed mice do not appear to have been overwhelmed by the additional bacteria, since there was a decrease in bacteria in the 3 h following infection (Fig. 2). The plasma infiltration into the lung that occurs following exposure to phosgene might provide a better medium in the lung for the bacteria. However, edema, as indicated by increased lavage fluid protein, could not be demonstrated following a 4-h exposure to concentrations below 0.2 ppm (Hatch et al., 1986), and maximum lavage fluid protein concentration occur between 18–24 h after exposure. At higher concentrations (1 ppm) lung wet weights in rats were elevated for 7 days following exposure (Franch and Hatch, 1986). The fact that enhanced susceptibility to streptococci could not be demonstrated when infection was delayed by as little as 24 h argues against a major role for edema fluid. Antibacterial factors have been demonstrated in lung surfactant that promote both intracellular and extracellular bactericidal activity (Coonrod and Yoneda, 1983). It is possible that phosgene exposure somehow alters these factors and hence diminishes bactericidal activity. In addition, the ability to adhere to tissue, and hence avoid phagocytosis, is a virulence factor for bacteria (Ellen and Gibbons, 1972). Enhanced susceptibility to streptococci might be explained if phosgene somehow were to alter the lung surface such that even a few bacteria were able to attach to the tissue and grow. Finally, the effects of phosgene may be due to additive or synergistic effects on several host defenses, no one of which clearly dominates.

Natural killer cells (both blood-borne and lung-associated) appear to have a significant role in limiting B16 tumors during both the early blood-borne phase and during the extravasation and/or early postextravasation phase (Hanna, 1982; Wiltrout et al., 1985). Depressed lung-associated natural killer cell activity in rats has been demonstrated following a single 4-h exposure to 0.5 but not 0.1 ppm phosgene (G. R. Burleson, personal communication). However, the fact that in our studies repeated daily exposure immediately following tumor injection had little effect on tumor numbers in the lung argues against effects on natural killer cell activity as the source of increased susceptibility ob-

served when tumors were given after phosgene exposure. Alternately, phosgene may affect the lung capillary bed such that cells are more likely to filter into the lung, due either to decreased flow through the lung or increased permeability.

Whatever the mechanisms involved, it is clear that exposures to very low levels of phosgene enhance the susceptibility of mice to both lung bacterial infection and lung tumor formation. Susceptibility to influenza virus infection in the lung was largely unaffected by phosgene exposure. The data are particularly relevant because they indicate effects due to exposure well below the current TLV at levels that might conceivably be reached as a result of accidental release in industrial settings.

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